# SOME EFFECTS OF ULTRA-VIOLET RADIATION UPON THE CALCIUM AND PHOSPHORUS CONTENT OF HIGHER PLANTS

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## I. REVIEW OF PREVIOUS WORK AND STATEMENT OF THE PROBLEM

That ultra-violet radiation exerts an accelerative effect upon calcium and phosphorus metabolism, especially the former, has been demonstrated repeatedly. Among the numerous workers who have made studies in the field are these: Steenbock and Nelson ('23), who showed that ultra-violet rays restore growth in rats deprived of fat-soluble vitamines; Orr, Holt, Wilkins, and Boone ('23), who demonstrated that ultra-violet rays cause large amounts of calcium and phosphorus to be retained in the body; Vignard, Mouriquand, Chassard and Bernheim ('23), who brought forth radiographic evidence that ultra-violet promotes the precipitation of calcium at the junctures of bone and cartilage; Clark ('23), who showed that the diffusible calcium of the blood is higher after the serum is exposed to ultra-violet radiation; Grant and Gates ('24), who found that the blood calcium of irradiated rabbits increases considerably over that of controls. Other workers in the field have been Huldschinsky, Hess, Powers, Funk, and Park ('23), the latter of whom gives an inclusive review of the literature concerning the effects of radiant energy on rickets.

The effects of a similar treatment with ultra-violet radiation upon the calcium and phosphorus content of higher plants seemed to the authors to constitute a problem in comparative physiology worthy of investigation, because thereby an additional contribution might be made to the long list of physiologic analogies between plants and animals.

In a survey of the literature concerning the effect of ultra-violet radiation upon plants, evidence of previous work upon this question has been almost entirely lacking. Indeed it seems that Beeskow ('27) has been the only investigator to report upon the effects of ultra-violet rays on the calcium and phosphorus of plants. His work, mentioned briefly at Nashville, appeared to show that rayed plants of *Zea Mays* exhibited increased calcium and phosphorus content. In the present paper the authors have attempted to present additional and more complete data concerning this particular aspect of ultra-violet physiology.

## II. MATERIAL

The plant material used in this work consisted of tomatoes and cucumbers, as employed in a previous work (Fuller, '31) on the stimulatory effects of radiation from a quartz mercury vapor arc. In that work plants were rayed according to various schedules with different screens. Of these groups, one, rayed daily for five weeks with Vita-glass at one hundred inches from the arc, showed definitely accelerated growth as compared with the controls, which received no ultra-violet radiation; the rayed and control sets were designated respectively as E and A. In comparison with the controls the plants rayed under Vita-glass were nearly one-third taller at the end of the experiment, showed a slightly increased dry weight and ash content, and were in every respect extremely healthy. Dry powders of these plants were used for the analytic work which is described below.

## III. METHODS

Because of the varying analytical results obtained from different procedures, the methods used in this work are described in detail, even at the risk of repeating information already present in chemical literature.

*Preparation of sample.*—One-gram portions of the powdered, air-dried sample were thoroughly ashed by a Fischer burner and the residue dissolved in the crucible with 2 cc. of concentrated hydrochloric acid. The contents of the crucible were then washed into a porcelain casserole with about 50 cc. of dilute hydrochloric acid (1 : 3) and evaporated to dryness on the water bath; the residue was baked two hours in an electric oven at 120° C. to render the silica insoluble. To the residue was then added 150 cc. of dilute hydrochloric acid, after which the casserole was allowed to stand on the water bath half an hour to insure the complete dissolving of the soluble constituents. Silica was removed by filtering through a hard filter-paper. The entire silica-free filtrate prepared from each gram of air-dried sample was used in a single determination of calcium or phosphorus in order to avoid the labor of preparing exact volumetric aliquots of a single prepared solution.

*Estimation of calcium.*—Calcium was estimated by titration with .1 N potassium permanganate according to the method of McCrudden ('09) and Mitchell ('21). Previous experience of the authors has shown this method to be susceptible of extraordinary accuracy, the limit of which is determined largely by the accuracy with which the original samples are taken. The prepared solution from one gram of air-dried sample was transferred to a 300-cc. beaker and made up to a volume of about 200 cc. Two drops of methyl orange were added and the solution made slightly alkaline with ammonium hydroxide (1 : 1). Dilute hydrochloric acid was then added drop by drop with constant stirring until the indicator showed a faintly acid reaction. Then 10 cc. of .5 N hydrochloric acid and 10 cc. of a 2.5 per cent solution of oxalic acid were added. The mixture was boiled, and 20 cc. of a saturated solution of ammonium oxalate added slowly with constant stirring. The mixture was heated until the precipitate became sufficiently granular for filtration, then cooled, and 8 cc. of a 20 per cent solution of sodium acetate (or enough to bring the solution to an alkaline reaction) were added. After standing over night the calcium oxalate was removed by filtration and washed with hot water until free from chlorides. The filter-paper was ruptured with a stirring rod, and the residue washed with hot water into the original beaker in which the calcium oxalate was precipitated. The precipitate was dissolved by the addition of 10 cc. of sulphuric acid (1 : 1) to the hot mixture. The hot solution was titrated immediately with .1 N potassium permanganate.

*Estimation of phosphorus.*—Phosphorus was estimated by precipitating with molybdate and weighing as magnesium pyrophosphate as described in "Official and Tentative Methods of Analysis" of the Association of Official Agricultural Chemists ('24). The prepared solution from one gram of air-dried sample was made up to about 50 cc. volume with distilled water. Concentrated ammonium hydroxide was added drop by drop with constant stirring until a slight precipitate was formed. This precipitate was dissolved by a few drops of concentrated nitric acid. Since hydrochloric acid had been used as a solvent for the ash, about 15 grams of dry ammonium nitrate were added. The solution was heated, and 40 cc. of molybdate solution were added. The mixture was digested an hour on the water bath, filtered, and the residue washed with dilute ammonium nitrate. The precipitate was dissolved on the filter-paper with ammonium hydroxide (1 : 1) and the paper washed with hot water until the volume of solution and washing was about 100 cc.

Hydrochloric acid (1 : 3) was added drop by drop until only a faint odor of ammonia remained, and the solution cooled in the Kelvinator. To the chilled solution 10 cc. of magnesia mixture were added by means of a burette, drop by drop, with vigorous stirring. After 15 minutes 10 cc. of concentrated ammonium hydroxide were added, and after standing over night the precipitate was filtered on an ashless filter-paper and washed free from chlorides. It was then ignited with a Fischer burner to a constant weight of magnesium pyrophosphate.

TABLE I

STIMULATORY EFFECTS AS EXEMPLIFIED BY WEIGHT DATA

| Plant | Average wet weight per plant in gms. | | Average dry weight per plant in gms. | | Average ash % of dry weight | |
|---|---|---|---|---|---|---|
| | Control A | U-V. E | Control A | U-V. E | Control A | U-V. E |
| Cucumber | 11.75 | 14.16 | 1.059 | 1.423 | 18.02 | 20.29 |
| Tomato | 10.52 | 15.18 | .8489 | 1.586 | 16.98 | 19.15 |

TABLE II

DATA ON PHOSPHORUS ANALYSES

| | 1 | | 2 | | 3 | 4 | |
|---|---|---|---|---|---|---|---|
| Plant | % $P_2O_5$ of dry weight | | Average uptake of $P_2O_5$ per plant in gms. | | Average % increase of $P_2O_5$ per plant of the rayed plants | Average % decrease of $P_2O_5$ in dry weight in rayed plants | |
| | Control A | U–V. E | Control A | U–V. E | | Actual decrease (A–E) | Relative decrease $\left(\frac{A-E}{A} \times 100\right)$ |
| Cucumber | 1.498±.034 | 1.385±.043 | .0159 | .0197 | 23.89 | .113 | 7.54 |
| Tomato | 1.146±.022 | .9865±.025 | .0097 | .0156 | 60.82 | .159 | 13.87 |

Explanation of tables.—Column 3 represents the average per cent increase of the total uptake in grams of $P_2O_5$ per plant. Column 4 represents two phases of the results relative to dry weight. The first, the actual difference, represents the algebraic difference between the controls and the rayed sets; the second, the percentage of this difference.

TABLE III

DATA ON CALCIUM ANALYSES

| | 1 | | 2 | | 3 | 4 | |
|---|---|---|---|---|---|---|---|
| Plant | % CaO of dry weight | | Average uptake of CaO per plant in grams | | Average % increase of CaO per plant of the rayed plants | Average % increase of CaO in dry weight | |
| | Control A | U-V. E | Control A | U-V. E | | Actual increase (E–A) | Relative increase $\left(\frac{E-A}{A} \times 100\right)$ |
| Cucumber | 4.239 ± .048 | 4.596 ± .088 | .0449 | .0654 | 45.43 | .357 | 8.44 |
| Tomato | 2.769 ± .022 | 2.824 ± .012 | .0235 | .0448 | 90.64 | .055 | 1.98 |

## IV. DISCUSSION

From the tables it is obvious that, first, the calcium content of the rayed tomato and cucumber plants is greater than that of the unrayed plants, and second, the phosphorus content of the rayed sets is lower than that of the controls. The results concerning calcium, then, support the findings of Beeskow and show an interesting similarity to the physiologic effects of ultra-violet radiation on animal tissue. As to the results of the phosphorus analyses, however, the condition is reversed—the rayed plants show the lower content, a condition contrary to that found by Beeskow and to that obtaining in animal tissue subjected to ultra-violet. The actual phosphorus *uptake* of the rayed plants is larger, however, than that of the controls, as is shown in column 3, table II, since the rayed plants show a greater amount of growth; but the actual percentage of phosphorus in the latter plants is lower than that of the controls.

No attempt is made in this paper to present an explanation of these phenomena concerning calcium and phosphorus, since data requisite to such an explanation, particularly information about phytosterol activity and vitamine potency, are lacking. The paper does, however, emphasize the definite calcium increase.

## V. SUMMARY

1. Tomato and cucumber plants which had been stimulated to greater growth by ultra-violet radiation showed a definite increase in calcium content, calculated as percentage of dry weight.

2. The same plants showed a decrease in phosphorus content, determined in the same manner.

3. The analytic procedure is described in detail.

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BIBLIOGRAPHY

Association of official agricultural chemists. ('24). Official and tentative methods of analysis. ed. 2. 1924.

Beeskow, H. C. ('27). Some physiological actions of ultra-violet rays on plants. Paper at Am. Assoc. Adv. Sci. Plant Physiol. Sect. Nashville, Dec. 1927.

Clark, J. ('23). The effect of ultra-violet light on the condition of Ca in the blood. Am. Jour. Hyg. **3:** 481. 1923.

Fuller, H. J. ('31). Stimulatory effects of radiation from a quartz mercury vapour arc upon higher plants. Ann. Mo. Bot. Gard. **18:** 17–40. 1931.

Grant, J. H., and Gates, F. L. ('24). A preliminary survey of the effects of ultra-violet light on normal rabbits. Soc. Exp. Biol. and Med. Proc. **21:** 230. 1924.

McCrudden, F. H. ('09). The quantitative separation of Ca and Mg in the presence of phosphates and small amounts of iron devised especially for the analysis of foods, urine, and feces. Jour. Biol. Chem. **7:** 83–100. 1909.

Mitchell, J. H. ('21). Report on inorganic plant constituents. Assoc. Off. Agr. Chem. Jour. **4:** 391–394. 1921.

Orr, W. J., Holt, L. E. Jr., Wilkins, L., and Boone, F. H. ('23). The Ca and P metabolism in rickets with special reference to ultra-violet ray therapy. Am. Jour. Diseases of Children **26:** 362–372. 1923.

Park, E. A. ('23). The etiology of rickets. Physiol. Rev. **3:** 106. 1923.

Steenbock, H., and Nelson, E. M. ('23). Light in its relation to ophthalmia and growth. Jour. Biol. Chem. **56:** 355–373. 1923.

Vignard, Mouriquand, Chassard and Bernheim ('23). Influence des rayons ultra-violets sur la precipitation du Ca dans les os rachitques. Lyon Méd. **32:** 1021. 1923.